Caenibacterium thermophilum is a later synonym of *Schlegelella thermodepolymerans*

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Recently, two strains of *Schlegelella thermodepolymerans* Elbanna *et al*. 2003 and an independently isolated bacterium, *Caenibacterium thermophilum* Manaia *et al*. 2003, were described in parallel as gen. nov., sp. nov. Analysis of the 16S rRNA genes revealed similarity between *C. thermophilum* and the two strains of *S. thermodepolymerans* of 99·8 and 99·6 %, respectively. DNA–DNA hybridization experiments revealed mean DNA reassociation levels of 97–98 % among *C. thermophilum* and the two strains of *S. thermodepolymerans*, thereby confirming the close relationship and indicating that *C. thermophilum* is a later synonym of *S. thermodepolymerans*.

Many bacteria from diverse taxonomic groups accumulate polyhydroxyalkanoates (PHAs) as storage compounds for carbon and energy under unbalanced growth conditions (Schlegel *et al*., 1961; Anderson & Dawes, 1990). The most abundant type of PHA is poly(3-hydroxybutyrate), which was described in 1926 (Lemoigne, 1926); now, more than 140 different PHA constituents are known (Steinbüchel & Valentin, 1995). Because of the beneficial material properties of PHAs, they have attracted a lot of interest from academia and industry in the context of a variety of technical applications (Steinbüchel, 1991; Hocking & Marchessault, 1994). In general, PHAs are water-insoluble, thermoplastic and/or elastomeric, enantiomerically pure, non-toxic, biocompatible and, in particular, biodegradable (Doi & Steinbüchel, 2001).

Naturally, PHAs are released to the environment after cell lysis, and a large variety of PHA-utilizing micro-organisms occur in many ecosystems such as soil or compost (Delafield *et al*., 1965; Jendrossek *et al*., 1996). PHA-degrading micro-organisms excrete specific hydrolysing enzymes; many of these extracellular PHA depolymerases have been genetically and biochemically characterized (Jendrossek & Handrick, 2002). However, most studies have involved mesophilic micro-organisms and enzymes, so little is known about the biodegradation of PHAs at elevated temperatures.

Takeda *et al*. (1998) were the first to report on a thermotolerant and thermostable PHA depolymerase from a bacterium growing optimally at 45–50 °C. A thermophilic strain, HS^T, was isolated from hot-spring water and initially affiliated to the genus *Leptothrix* (Takeda *et al*., 1998, 2000). Later, it was taxonomically characterized in detail and described as *Caldimonas manganoxidans* gen. nov., sp. nov. (Takeda *et al*., 2002).

In a recent study, we isolated a novel bacterium from activated sludge under thermophilic growth conditions that was characterized as *Schlegelella thermodepolymerans* gen. nov., sp. nov. (Elbanna *et al*., 2003). This strain was capable of degrading poly(3-hydroxybutyrate) as well as the 3-mercaptopropionate-containing copolymer poly(3-hydroxybutyrate-*co*-3-mercaptopropionate). The latter was synthesized by PHA-accumulating bacteria, representing the first example of a novel class of biopolymer, which was referred to as polythioester (Lütke-Eversloh *et al*., 2001; Lütke-Eversloh & Steinbüchel, 2004). Furthermore, the extracellular thermostable PHA depolymerase of *S. thermodepolymerans* was purified and biochemically characterized: it was found to have substrate specificity for the oxoester linkages of PHAs, because thioesters could not be hydrolysed by this type of enzyme (Elbanna *et al*., 2004).

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Abbreviation: PHA, polyhydroxyalkanoate.

Interestingly, the polyester-degrading bacterium *Caenibacterium thermophilum* gen. nov., sp. nov., which was isolated from a thermophilic municipal sludge digester, was investigated at the same time, in parallel, in a different laboratory (Manaia *et al.*, 2003). Therefore, *Caenibacterium thermophilum* and *S. thermodepolymerans* could be compared phylogenetically only after the published data became available (presented here). Both independently isolated bacteria showed significant similarities and, because of the date of valid publication of the names, we propose that *Caenibacterium thermophilum* is a later synonym of *S. thermodepolymerans*.

The 16S rRNA gene sequences were analysed by using the program BLAST (National Center for Biotechnology Information, http//www.ncbi.nlm.nih.gov). The consensus sequences of strains belonging to the same phylogenetic group and of other representatives of the *β-Proteobacteria* (retrieved from the EMBL database) were aligned using the computer program CLUSTAL_X (Thompson *et al.*, 1997). The resulting phylogenetic tree was calculated using the neighbour-joining method (Saitou & Nei, 1987) and displayed with TreeView (Page, 1996), using *Wautersia metallidurans* as an outgroup. Analysis of the 16S rRNA gene sequence of *Caenibacterium thermophilum* N2-680^T revealed 99·8 and 99·6 % similarity to the 16S rRNA gene sequences of strains K14^T and DhA-71, respectively, of *S. thermodepolymerans*, indicating that these bacteria belong to the same species. Regarding the nearest phylogenetic neighbours of the *β*-subclass of the *Proteobacteria*, *S. thermodepolymerans* is related to *Leptothrix*, *Rubrivivax*, *Ideonella*, *Roseateles* and *Aquabacterium* (Fig. 1). Interestingly, *S. thermodepolymerans* exhibited 94 % similarity to *Caldimonas manganoxidans*, a recently described thermophilic bacterium that also degrades poly(3-hydroxybutyrate) (Takeda *et al.*, 2002).

DNA–DNA hybridizations were carried out with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989), using an HTS7000 Bio Assay Reader (Perkin Elmer) measuring fluorescence. The hybridization temperature was 50 °C. Reciprocal experiments were performed for every pair of strains and the values were interpreted according to the recommendations of Wayne *et al.* (1987). DNA–DNA hybridization confirmed that strain N2-680^T is closely related to K14^T and DhA-71, there being mean DNA reassociation levels of 97–98 % among the three strains.

Emended description of *Schlegelella thermodepolymerans*

Synonym *Caenibacterium thermophilum* Manaia *et al.* 2003.

Gram-negative, non-pigmented, non-spore-forming, aerobic, rod-shaped cells, 1·0–2·8 μm long and 0·5–0·6 μm wide, motile by means of polar monotrichous flagellation. Growth occurs between pH 6 and 9. The predominant fatty acids are $C_{16:0}$ and cyclo-$C_{17:0}$; the hydroxylated fatty acids 3-OH-$C_{10:0}$ and 3-OH-$C_{12:0}$ are present. Details for

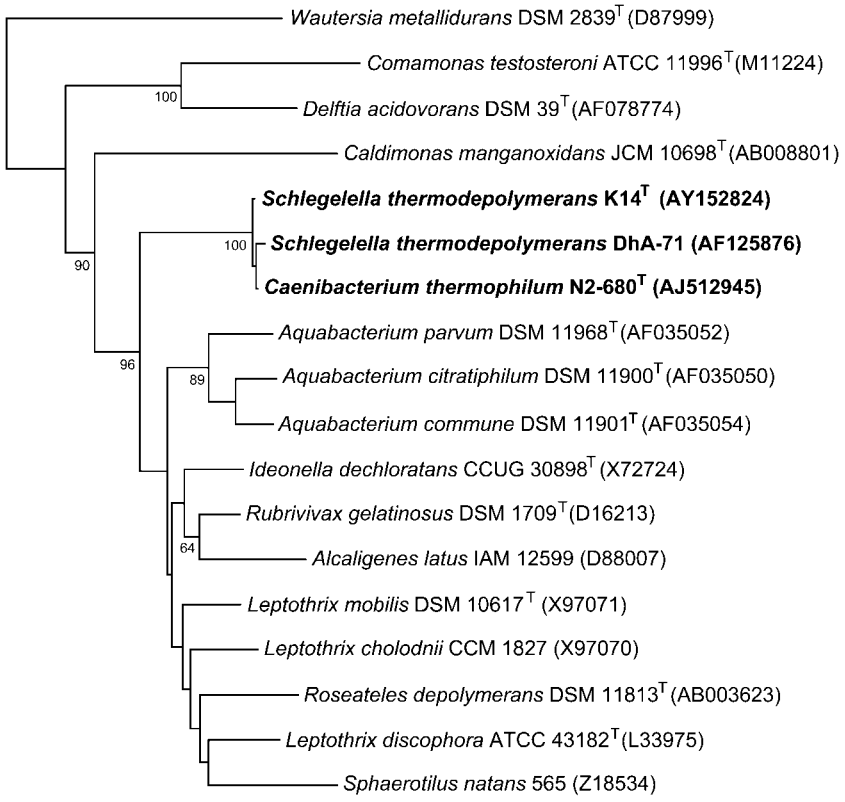

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the estimated phylogenetic relationships among *S. thermodepolymerans*, *Caenibacterium thermophilum* and the nearest members of the *β-Proteobacteria*. Accession numbers are given in parentheses. Bootstrap values are shown for relevant branches as percentages of 1000 replicates. Bar, 1 % sequence divergence.

specific strains can be found in Elbanna *et al.* (2003) and Manaia *et al.* (2003).

The type strain is K14^T (=LMG 21644^T=DSM 15344^T).

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